

Analytical, Nutritional and Clinical Methods Section

Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions

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Abstract

This study on anthocyanin stability and colour variation (λ_{\max} , ϵ) in the pH range 1–12 during a period of 60 days storage at 10 and 23°C, was conducted on the 3-glucosides of the six common anthocyanidins. It was mostly in the alkaline region that differences in colour and stability became significant. Although it has been generally accepted that anthocyanins are stable only at low pH values, this study revealed that, for some of the anthocyanin 3-glucosides (e.g. malvidin 3-glucoside), the bluish colours were rather intense and stability relatively high in the alkaline region. Thus, they can be regarded as potential colorants for some slightly alkaline food products. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The anthocyanins form the red and blue colours of most fruits and vegetables and provide, therefore, the attractive colours of many fruit juices, wines, jams and preserves. There is worldwide interest in additional use of anthocyanins as a consequence of perceived consumer preferences as well as legislative action, which has continued the delisting of approved artificial dyes. Nevertheless, the anthocyanin colours are easily affected by a number of factors (Brouillard, 1988; Francis, 1989; Fossen, Cabrita & Andersen, 1998).

The aim of this paper is to describe the colour and stability changes of the six common anthocyanidin 3-monoglucosides in aqueous solutions at different pH values and during storage at two different temperatures. In a recent paper, similar parameters were described for cyanidin 3-*O*- β -glucoside and petunidin 3-[6-*O*-(4-*p*-coumaroyl- α -rhamnosyl)- β -glucoside]-5-*O*- β -glucoside (Fossen et al., 1998). In general, fruits contain simpler anthocyanins than flowers, and most of these anthocyanins are without acylation (Andersen, 1999). Thus, the colour behaviour of the six anthocyanidin 3-glucosides examined in this study may represent the majority of the anthocyanins isolated from fruits, as for instance identified in chokeberries (*Aronia* spp.), strawberries

(*Fragaria* spp.), apples (*Malus* spp.), cherries and plums (*Prunus* spp.), currants (*Ribes* spp.), raspberries and blackberries (*Rubus* spp.), elderberries (*Sambucus* spp.), cranberries and blueberries (*Vaccinium* spp.), etc. (Mazza & Miniati, 1993).

2. Materials and methods

2.1. Anthocyanin sources

Pigments were obtained from the following sources: strawberry (*Fragaria ananassa*: pelargonidin 3-glucoside, pg3glc); rice (*Oriza sativa*: cyanidin and peonidin 3-glucosides, cy3glc and pn3glc); *Abies koreana* (delphinidin and petunidin 3-glucosides, dp3glc and pt3glc); blueberries (*Vaccinium* spp.: malvidin 3-glucoside, mv3glc).

2.2. Pigment isolation

The plant materials were extracted with 0.2% HCl in MeOH at 4°C, and the extracts were concentrated under reduced pressure. The crude extracts were partitioned against hexane and ethyl acetate, and washed on a XAD-7 Amberlite column. Pigments were purified by DCCC (Droplet Counter-Current Chromatography) using BAW (4:1:5) upper and lower phases as stationary and mobile phases, respectively, and size-exclusion

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chromatography (Sephadex LH-20 column, 100×1.6 cm, Pharmacia) using H_2O -MeOH-HCl (60:40:0.05, v/v) as eluent. Some pigments were further purified by semi-preparative RP-HPLC (reversed-phase High Performance Liquid Chromatography) using a gradient elution system consisting of solvents A: water-formic acid (9:1) and B: water-formic acid-methanol (4:1:5) (Cabrita & Andersen, in press). The flow-rate was 5 ml/min. Pigment identity was confirmed by proton and carbon NMR spectroscopy.

2.3. Buffer solutions

Buffer solutions of 17 different pH values were prepared in accordance with Table 1. The accurate pH values for each buffer solution were measured with a HANNA HI 9224 portable pH Meter equipped with a HI 1230B Combination pH Electrode and a HI 7669/2W Temperature Probe. The spectral behaviour of anthocyanins is dependent on solvent, and substances present in the solutions may influence the colour and stability.

2.4. Measurements of colour and stability

The colour stability of the pure anthocyanin 3-glucosides was determined at 10 and 23°C, respectively. The chloride salts of the isolated pigments were dissolved in methanol containing 0.05% conc. HCl to make stock solutions with concentration 0.5 mg/ml, and 2 ml aliquots of these solutions were transferred to sample tubes, evaporated to dryness and dissolved in 20 ml of each of the 17 different buffer solutions (Table 1) to give a final pigment concentration of 0.05 mg/ml. Each anthocyanin buffer solution was divided into two 10 ml

portions which were stored at 10 and 23°C, respectively. The sample tubes were sealed with parafilm, and the pigments were stored under air atmosphere. UV/Vis spectra were recorded between 240 and 700 nm on a VARIAN CARY3 UV-Visible Spectrophotometer equipped with a 386 ACER 1120SX computer unit. Pure buffers were used as reference cell solutions. UV/Vis measurements were made 1h after dissolution and then after 1, 2, 5, 8, 15 and 60 days. The colour intensities were measured as absorbance values at $\lambda_{\text{vis-max}}$ for each individual pigment at each pH value and expressed as molar absorptivity (ϵ , in $\text{M}^{-1} \text{cm}^{-1}$). Colour stability was expressed as the percentage of absorbance remaining after a certain time interval, measured at $\lambda_{\text{vis-max}}$ for individual pigments at each pH value.

3. Results and discussion

3.1. Colour variation of anthocyanin 3-glucosides at pH 1–12

By plotting the $\lambda_{\text{vis-max}}$ values (Table 2) obtained for the six common anthocyanidin 3-glucosides (Fig. 1) 1 h after dissolution in aqueous solutions at different pH values, a distinct pattern was achieved. The anthocyanins could be placed into two groups based on their curve shapes. Group 1, which consisted of the 3-glucosides of pelargonidin, peonidin and malvidin, was described by parallel curves showing gradual bathochromic shifts up to pH 6 (Fig. 2). In the pH region 6.0 to 7.6 the bathochromic shifts increased markedly, but the bluish colours would not further change above pH 8. Group 2, which contained the 3-glucosides of cyanidin, petunidin and delphinidin, revealed similar curves as members of

Table 1
Solvent proportions (v/v) used to prepare 17 different buffer solutions in the pH region 1–12

pH	KCl 0.2 M	KH phthalate 0.1 M	KH_2PO_4 0.1 M	BORAX 0.025 M	Na_2HPO_4 0.05 M	HCl 0.02 M	HCl 0.1 M	NaOH 0.1 M
1.0	25					67		
2.4		50					42.2	
3.1		50					18.8	
4.0		50					0.1	
5.0		50						22.6
6.0			50					5.6
6.5			50					13.9
7.0			50					29.1
7.3			50					37.0
7.7			50					43.5
8.1			50					46.1
8.6				50			13.5	
9.0				50			4.6	
9.5				50				8.8
9.8				50				15.0
10.6				50				23.3
11.5					50			11.1

Table 2

Visible λ_{max} values in nm for chloride salts of the six common anthocyanidin 3-glucosides (1.0×10^{-4} M) one hour after dissolution in buffered aqueous solutions at different pH values and room temperature

pH	Pg3glc	Cy3glc	Pn3glc	Dp3glc	Pt3glc	Mv3glc
1.0	498	510	510	514	515	517
2.4	501	512	516	521	521	522
3.1	504	517	518	525	525	528
4.0	507	520	522	528	529	533
5.0	515	523	527	530	531	535
6.0	519	528	532	558	565	537
6.5	525	539	537	567	569	559
7.0	540	554	554	576	584	576
7.3	547	562	568	574	589	586
7.7	551	571	571	577	590	593
8.1	553	570	574	574	588	594
8.6	553	539	571	542	543	595
9.0	555	540	573	547	542	596
9.5	554	542	573	552	543	597
9.8	553	541	573		546	598
10.6	554	569	575			595
11.5						588

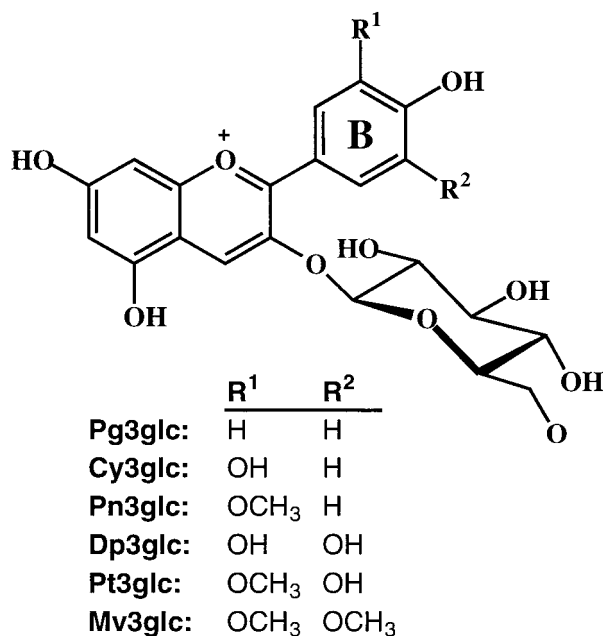


Fig. 1. Structure of the six most common anthocyanidin 3-mono-glucosides.

Group 1 in the pH region 1.0 to 8.1 (Fig. 2). However, all members of this latter group showed dramatic hypsochromic shifts going from pH 8.1 to 8.6.

It is well known that an increase in the number of oxygen substituents (hydroxyl or methoxyl) on the anthocyanidin B-ring causes bathochromic shifts at relatively acid pH values (Harborne, 1993; Torskangerpoll, Børve, Andersen & Sæthre, 1998). Fig. 2 shows that the same tendency occurs at all pH values for the 3-glucosides of pelargonidin, peonidin and malvidin with increasing numbers of methoxyl groups on the aglycone B-ring. For the 3-glucosides of cyanidin, delphinidin

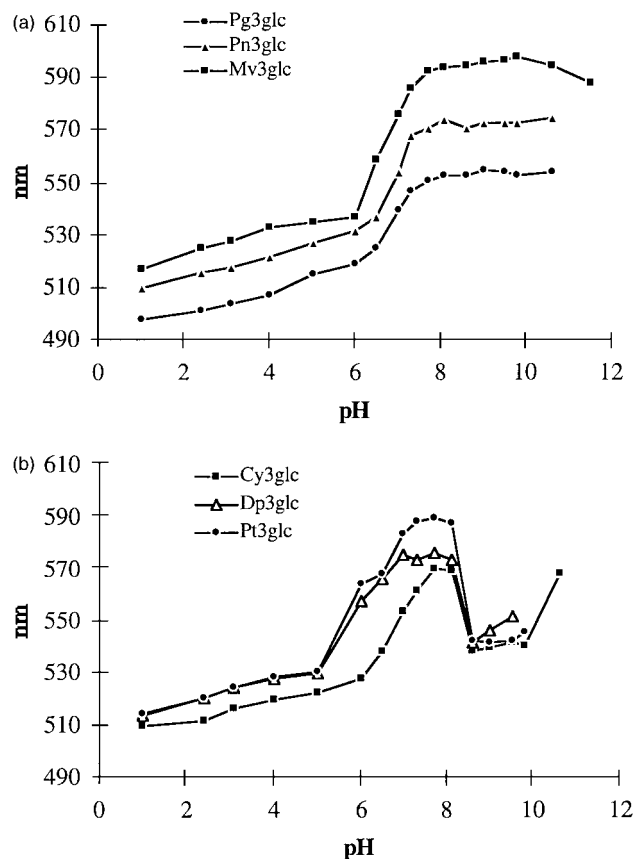


Fig. 2. Visible absorption maxima ($\lambda_{\text{vis-max}}$, nm) at different pH values, for the chloride salts of the six common anthocyanidin 3-glucosides (1.0×10^{-4} M) 1 h after dissolution in buffered aqueous solutions at room temperature.

and petunidin, the same bathochromic shift trend is shown from pH 1.0 to 8.1 with increasing number of hydroxyl and/or methoxyl groups in the B-ring. Thus, the hydroxyl and methoxyl groups on the anthocyanidin B-ring seem to have similar effects on the chromophores of the bluish equilibrium forms, as previously reported for anthocyanins when occurring in their flavylium forms (Brouillard, 1988).

3.2. Colour intensity of anthocyanin 3-glucosides at pH 1–12

The pH variation affects the colour intensities of the six anthocyanidin 3-glucosides considerably (Table 3). The absorptivities were highest at pH 1 for all pigments and decreased toward pH 5. The similarity between the curves in this pH region (Fig. 3) indicates that all the examined anthocyanins have the same type and distribution of equilibrium forms: the colourful flavylium form dominates at pH 1, and the occurrence of colourless carbinol pseudo-base forms increases toward pH 5 (Brouillard, 1988). At pH values above 6, anthocyanins show a hyperchromic effect until local maxima are achieved at pH 8.1–9.8, when anthocyanins are expected

to occur mainly in their quinonoidal and quinonoidal anion forms.

Fig. 3 reveals different behaviour for the individual anthocyanins around neutrality and especially in alkaline solutions. The ratio between the absorptivity at the local maximum in the alkaline region and at pH 1 for the 3-glucosides of pelargonidin, cyanidin, delphinidin and petunidin are found between 0.60 and 0.66. The ratios for peonidin 3-glucoside and malvidin 3-glucoside were 0.82 and 0.94, respectively, indicating that a B-ring substitution pattern which includes no hydroxyl groups in *ortho*-positions to each other and one or more methoxyl groups, is favourable for colour intensity in the alkaline region. However a comparison between the molar absorptivities of petanin, petunidin 3-[6-*O*-(4-*p*-coumaroyl- α -rhamnosyl)- β -glucoside]-5-*O*- β -glucoside

Table 3

Molar absorptivities for the chloride salts of the six common anthocyanidin 3-glucosides (1.0×10^{-4} M) 1 h after dissolution in buffered aqueous solutions at different pH values and room temperature

pH	Pg3glc	Cy3glc	Pn3glc	Dp3glc	Pt3glc	Mv3glc
1.0	14 300	20 000	15 100	13 000	21 300	23 400
2.4	11 600	18 400	13 000	10 900	18 400	20 500
3.1	8600	14 600	10 000	8400	13 900	14 000
4.0	3900	6800	4500	3600	5900	5900
5.0	1500	1600	1600	1300	1500	1800
6.0	1300	2000	1500	2600	4600	1300
6.5	1700	4200	2400	3200	5500	2700
7.0	4700	7000	5200	5700	7300	8400
7.3	5300	8400	6300	6200	8800	10 200
7.7	6400	10 700	8300	5600	11 500	13 200
8.1	7300	12 500	10 200	4500	13 400	16 400
8.6	8400	10 100	10 500	5400	10 200	19 500
9.0	9500	10 500	11 500	7800	10 300	22 000
9.5	8800	11 900	12 400	2800	10 300	13 000
9.8	8500	11 100	12 400		9800	13 000
10.6	6600	7100	9600			10 500
11.5						1100

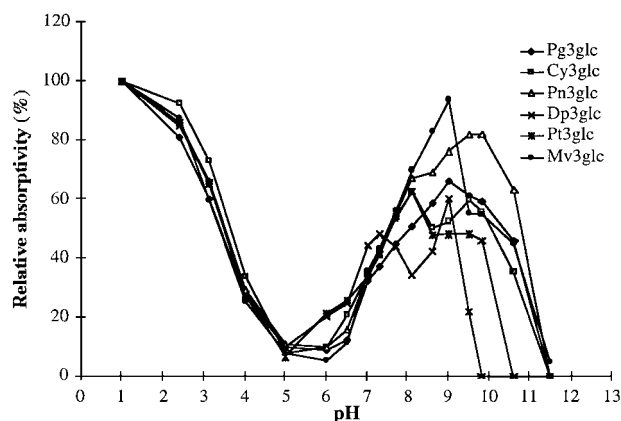


Fig. 3. Absorptivities relating to pH 1 values for the chloride salts of the six common anthocyanidin 3-glucosides (1.0×10^{-4} M) 1 h after dissolution in buffered aqueous solutions at room temperature.

(Fossen et al., 1998) and petunidin 3-*O*- β -glucoside, shows that a complex anthocyanin, like petanin, has much higher colour intensity than anthocyanidin 3-monoglucosides in solutions with pH between 6.0 and 8.6.

3.3. Anthocyanin stability on storage at 10 and 23°C, pH 1–12

All examined anthocyanins showed stability above 70% after 60 days at pH 1–3 and 10°C, and lower stability values at higher pH values (Table 4). After 8 days of storage it was found that stability decreased rapidly as pH increased toward pH 5–6. However, the stability of some anthocyanins improved as pH increased into the alkaline region, and local stability maxima were achieved around pH 8–9 (Fig. 4). Anthocyanins, such as pg3glc, pn3glc and mv3glc (Group 1), displayed 30–60% stability after 8 days at these pH values, and mv3glc and pg3glc kept 21–37% of their initial absorbance after 15 days storage. Dp3glc and pt3glc behaved quite differently under similar conditions (8 days storage at 10°C). In the pH 5–7 range, they exhibited higher stability than anthocyanin 3-glucosides belonging to Group 1 (Fig. 4). In fact, these anthocyanins are the only ones showing some degree of stability in this pH range, although the corresponding colour intensities are modest. On the other hand, they are very unstable at nearly all alkaline values (Table 4). At pH 6–7, dp3glc and pt3glc formed purplish precipitates, which were suspended upon shaking.

From a structural point of view, it seems that the presence of only one free hydroxyl group in the B-ring

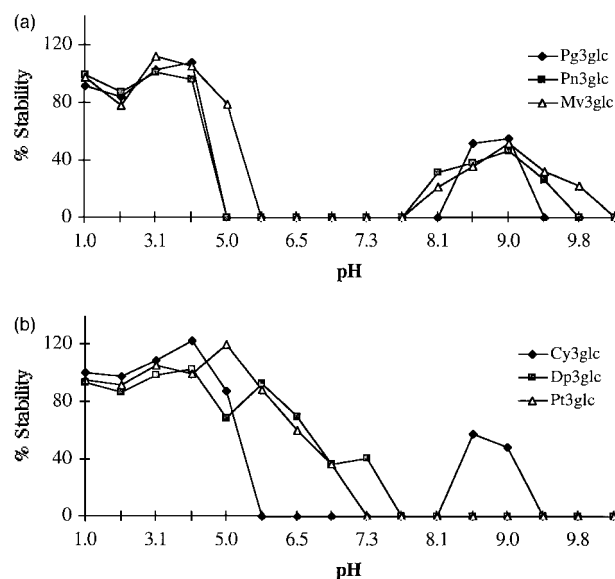


Fig. 4. Percentages of remaining absorbance (measured at $\lambda_{\text{vis-max}}$) after 8 days of storage at 10°C in buffered aqueous solutions at different pH values, for the chloride salts of the six common anthocyanidin 3-glucosides (1.0×10^{-4} M), in the dark and using air atmosphere.

Table 4

Absorbances for the chloride salts of the six common anthocyanidin 3-glucosides (1.0×10^{-4} M) measured during 60 days of storage at 10°C in buffered aqueous solutions from pH 1 to 12, in the dark in air atmosphere

	1.0	2.4	3.2	4.0	5.0	6.0	6.5	7.0	7.3	7.7	8.1	8.6	9.0	9.5	9.8	10.6	11.5
<i>pg3glc</i>																	
0	1.48	1.20	0.89	0.40	0.15	0.13	0.18	0.48	0.55	0.66	0.75	0.87	0.98	0.88	0.85	0.66	0.00
1	1.42	1.04	0.91	0.45	0.11	0.13			0.30	0.52	0.62	0.76	0.88	0.73	0.65		
2	1.38	1.02	0.89	0.44	0.12					0.38	0.51	0.68	0.80	0.60	0.49		
5	1.36	1.0	0.90	0.42	0.10						0.38	0.55	0.66	0.37			
8	1.36	1.01	0.92	0.43								0.45	0.54				
15	1.35	1.02	0.90	0.40									0.36				
60	1.31	0.85	0.76														
<i>cy3glc</i>																	
0	2.06	1.90	1.51	0.70	0.17	0.21	0.43	0.72	0.87	1.10	1.29	1.04	1.08	1.23	1.15	0.73	0.00
1	1.91	2.00	1.59	0.78	0.14	0.14	0.12	0.23	0.45	0.69	1.02	0.98	0.95	1.09	0.88		
2	2.03	1.87	1.57	0.78	0.13	0.17	0.19		0.31	0.52	0.79	0.87	0.92	0.88	0.65		
5	2.07	1.78	1.56	0.77	0.12							0.59	0.56	0.46			
8	2.08	1.87	1.65	0.86	0.15							0.60	0.53				
15	1.97	1.78	1.54	0.58													
60	2.20	1.66	1.35														
<i>pn3glc</i>																	
0	1.56	1.34	1.03	0.46	0.17	0.16	0.25	0.53	0.65	0.86	1.05	1.08	1.19	1.27	1.27	0.99	0.00
1	1.55	1.33	1.03	0.49	0.14				0.37	0.63	0.89	1.00	1.12	1.13	1.13	0.33	
2	1.53	1.20	1.04	0.48	0.12					0.46	0.75	0.75	1.01	1.00	0.90		
5	1.53	1.16	1.04	0.47	0.13						0.47	0.57	0.76	0.59	0.42		
8	1.54	1.16	1.04	0.44							0.33	0.41	0.56	0.33			
15	1.55	1.16	1.04	0.45													
60	1.57	1.12	1.02	0.21													
<i>dp3glc</i>																	
0	1.33	1.13	0.87	0.37	0.13	0.27	0.33	0.59	0.64	0.58	0.46	0.56	0.80	0.30	0.00	0.00	0.00
1	1.24	1.09	0.90	0.41	0.09	0.18	0.26	0.36	0.41	0.31	0.28	0.47	0.66				
2	1.25	1.04	0.86	0.42	0.10	0.30	0.25	0.32	0.29	0.22	0.24	0.40	0.47				
5	1.28	1.01	0.88	0.40	0.09	0.27	0.22	0.24	0.27	0.27							
8	1.25	0.99	0.86	0.38	0.09	0.25	0.23	0.22	0.26								
15	1.26	0.97	0.85	0.35		0.23	0.20	0.20	0.24								
60	1.26	0.87	0.76	0.16													
<i>pt3glc</i>																	
0	2.19	1.90	1.43	0.60	0.15	0.47	0.57	0.75	0.91	1.19	1.38	1.05	1.06	1.06	1.01	0.00	0.00
1	2.23	1.92	1.48	0.66	0.17	0.46	0.43	0.51	0.51	0.69	0.85	0.90	1.17	0.85	0.69		
2	2.12	1.82	1.51	0.68	0.19	0.45	0.40	0.43	0.40	0.39	0.41	0.73	0.77	0.40	0.32		
5	2.11	1.77	1.53	0.65	0.18	0.45	0.39	0.35	0.31			0.46	0.48				
8	2.11	1.75	1.52	0.60	0.18	0.42	0.35	0.28									
15	2.11	1.74	1.48	0.51		0.32											
60	2.11	1.60	1.34														
<i>mv3glc</i>																	
0	2.42	2.12	1.44	0.61	0.19	0.13	0.28	0.87	1.05	1.36	1.69	2.01	2.27	1.46	1.45	1.18	0.13
1	2.36	1.77	1.66	0.65	0.15			0.28	0.47	0.83	1.21	1.70	2.10	1.22	1.20	0.72	
2	2.38	1.68	1.59	0.63	0.15					0.53	0.87	1.41	1.89	0.89	1.00	0.33	
5	2.37	1.68	1.63	0.65	0.18					0.30	0.53	1.01	1.50	0.72	0.55		
8	2.38	1.66	1.61	0.64	0.15						0.35	0.73	1.18	0.47	0.32		
15	2.38	1.67	1.63	0.63	0.14							0.42	0.71	0.18	0.12		
60	2.39	1.64	1.59	0.53	0.14												

of the anthocyanin (Group 1) seems to favour the stability of the bluish equilibrium forms occurring at alkaline pH values, which is further enhanced by the presence of additional methoxyl groups. Such is not the case for anthocyanins containing three oxygen functions (methoxyl or hydroxyl) in the aglycone B-ring, where two hydroxyl groups are in *ortho* position to each other. Cy3glc, which also shares the *ortho*-hydroxyl feature,

seems, however, to have a stability behaviour more closely related to those anthocyanins belonging to Group 1 (Table 4).

After 8 days of storage at 23°C, the dependence of colour stability on pH followed the same pattern as observed at 10°C, but the corresponding stability values were lower (Table 5). Between pH 1.0 and 2.4 all anthocyanins could endure a 60 day storage and showed

Table 5

Absorbances for the chloride salts of the six common anthocyanidin 3-glucosides (1.0×10^{-4} M) measured during 60 days of storage at 23°C in buffered aqueous solutions between pH 1 and 12, in the dark in air atmosphere

	1.0	2.4	3.1	4.0	5.0	6.0	6.5	7.0	7.3	7.7	8.1	8.6	9.0	9.5	9.8	10.6	11.5
<i>pg3glc</i>																	
0	1.48	1.20	0.89	0.40	0.15	0.13	0.18	0.48	0.55	0.66	0.75	0.87	0.98	0.88	0.85	0.66	0.00
1	1.37	1.14	0.87	0.37	0.13	0.14					0.39	0.52	0.61				
2	1.26	1.13	0.87	0.37								0.32	0.39				
5	1.32	1.12	0.83	0.29													
8	1.32	1.11	0.78	0.20													
15	1.32	1.00	0.56														
60	1.27	1.01															
<i>cy3glc</i>																	
0	2.06	1.90	1.51	0.70	0.17	0.21	0.43	0.72	0.87	1.10	1.29	1.04	1.08	1.23	1.15	0.73	0.00
1	2.18	1.86	1.48	0.68	0.16	0.15	0.22	0.21	0.26	0.41	0.73	0.77	0.80	0.49			
2	2.07	1.87	1.48	0.67	0.15	0.14	0.22				0.46	0.59	0.60				
5	2.02	1.86	1.45	0.60													
8	2.01	1.82	1.42	0.48													
15	2.02	1.82	1.37	0.36													
60	1.90	1.52	0.83														
<i>pn3glc</i>																	
0	1.56	1.34	1.03	0.46	0.17	0.16	0.25	0.53	0.65	0.86	1.05	1.08	1.19	1.27	1.27	0.99	0.00
1	1.56	1.34	1.00	0.45	0.17					0.33	0.52	0.69	0.73	0.64	0.44		
2	1.55	1.33	1.01	0.46	0.14						0.29	0.54	0.45	0.31			
5	1.54	1.33	1.01	0.44								0.24					
8	1.56	1.32	0.99	0.38													
15	1.52	1.30	0.96	0.21													
60	1.47	1.23	0.39														
<i>dp3glc</i>																	
0	1.33	1.13	0.87	0.37	0.13	0.27	0.33	0.59	0.64	0.58	0.46	0.56	0.80	0.30	0.00	0.00	0.00
1	1.32	1.10	0.83	0.35	0.11	0.18	0.22	0.25	0.25								
2	1.30	1.09	0.82	0.32	0.13	0.18	0.19	0.21	0.22								
5	1.28	1.04	0.75	0.25		0.19											
8	1.27	1.01	0.69	0.20													
15	1.24	0.94	0.56														
60	0.99	0.46	0.19														
<i>pt3glc</i>																	
0	2.19	1.90	1.43	0.60	0.15	0.47	0.57	0.75	0.91	1.19	1.38	1.05	1.06	1.12	1.07	0.00	0.00
1	2.15	1.86	1.38	0.58	0.16		0.21										
2	2.12	1.84	1.37	0.56	0.15												
5	2.08	1.81	1.34	0.51													
8	2.08	1.77	1.25	0.35													
15	2.02	1.66	1.10														
60	1.84	1.19	0.35														
<i>mv3glc</i>																	
0	2.42	2.12	1.44	0.61	0.19	0.13	0.28	0.87	1.05	1.36	1.69	2.01	1.27	1.46	1.45	1.18	0.13
1	2.41	2.10	1.40	0.61	0.16					0.38	0.66	1.00	1.43	0.77	0.57		
2	2.39	2.10	1.38	0.57	0.17						0.34	0.56	0.86	0.43	0.27		
5	2.41	2.08	1.37	0.51	0.16							0.24	0.36				
8	2.37	2.06	1.33	0.42	0.17												
15	2.35	2.03	1.22	0.27													
60	2.20	1.78	0.55														

above 40% colour. Then stability became minimum from pH 6.0 to 7.7, and again local maxima stabilities were achieved around pH 8–9 but at more modest values than at 10°C. At these alkaline values, some pn3glc and mv3glc (12–22%) withstood 5 days storage; more pg3glc and cy3glc (28–57%) remained after 2 days, while all dp3glc and pt3glc degraded within 1 h.

4. Conclusions

The colours, intensity and stability of the six common anthocyanidin 3-glucosides changed significantly in the pH 1–12 range. In relatively strong acidic aqueous solutions (pH 1–3), all the anthocyanin 3-glucosides occurred in the most intense reddish colours, typical for

their flavylium forms, and were very stable even after 60 days of storage at 10°C. Anthocyanin stability and colour intensity then decreased towards neutrality (pH 5–7), while there was a gradual bathochromic shift to more bluish colours.

It was in the alkaline region that anthocyanin properties became more distinguishable. The 3-glucosides of pelargonidin, peonidin and malvidin (Group 1) afforded bluish-coloured solutions with intensity and stability maxima around pH 8–9. As a common structural feature, these anthocyanins contain only one free hydroxyl moiety in the aglycone B-ring. The 3-glucosides of cyanidin, delphinidin and petunidin (Group 2) contain *ortho*-hydroxyl groups in the B-ring. These latter pigments exhibited a notable hypsochromic effect above pH 8.1, when their bluish equilibrium forms were converted into more reddish ones. Dp3glc and pt3glc were very unstable in alkaline media, however, they showed some stability in the pH 5–7 range. The stability of cy3glc under alkaline conditions was similar to that of Group 1 anthocyanins.

It should be kept in mind that other factors not directly addressed in this study (buffer composition, concentration, etc.) may influence the pH-dependency of colours and stability of pure anthocyanins in aqueous media.

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